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Plasma-Serum Parvovirus B19 PCR Detection Kit — Product Insert

Product # 39500

Parvovirus B19 was the first known human virus in the family of parvoviruses. B19 is a non-enveloped, icosahedral virus that contains a single-stranded linear DNA genome. It is classified as an erythrovirus because of its capability to invade red blood cell precursors in the bone marrow. B19 virus causes a childhood rash called fifth disease or erythema infectiosum which is commonly called “slapped cheek” syndrome due to the appearance of erythema across the cheeks. The virus is primarily spread by infected respiratory droplets, but some cases of blood-borne transmission have been reported. Parvovirus infection in pregnant women is associated with hydrops fetalis due to severe fetal anemia, sometimes leading to miscarriage or stillbirth. The risk of fetal loss is about 10% if infection occurs before week 20 of pregnancy. There is also some evidence that intrauterine Parvovirus B19 infection leads to developmental abnormalities in childhood. Due to the serious nature of the virus, methods for its rapid diagnosis are desirable.

Principle of the Test

Norgen’s Plasma-Serum Parvovirus B19 PCR Detection Kit constituents a ready-to-use system for the isolation and detection of Parvovirus B19 using end-point PCR. The kit first allows for the isolation of total DNA, including viral DNA, from the plasma-serum samples using spin-column chromatography based on Norgen’s proprietary resin. The viral DNA is isolated free from inhibitors, and can then be used as the template in a PCR reaction for Parvovirus B19 detection using the provided Parvovirus B19 2x PCR Master Mix. The Parvovirus B19 2x PCR Master Mix contains reagents and enzymes for the specific amplification of a 322 bp region of Parvovirus B19. In addition, Norgen’s Plasma-Serum Parvovirus B19 PCR Detection Kit contains a second Mastermix, the Control 2x PCR Master Mix, which can be used to identify possible PCR inhibition and/or inadequate isolation via a separate PCR reaction with the use of the *PCR control (PCRC)* or *Isolation Control (IsoC),* respectively. The amplification and detection of either the Parvovirus B19 *Isolation Control (IsoC)* or the *PCR control (PCRC)* does not reduce the detection limit of the analytical Parvovirus B19 PCR. The kit is designed to allow for the testing of 24 samples.

Kit Components:

Component	Contents
Lysis Solution	30 mL
Binding Solution 1	6 mL
Binding Solution 2	6 mL
Wash Solution	22 mL
Elution Buffer	3 mL
Mini Filter Spin Columns	24
Collection Tubes	24
Elution tubes (1.7 mL)	24
Parvovirus B19 2x PCR Master Mix	0.35 mL
Control 2x PCR Master Mix	0.35 mL
Isolation Control (IsoC)[a]	0.4 mL
Parvovirus B19 Positive Control (PosC)[b]	0.1 mL
Nuclease-Free Water	1.25 mL
Norgen’s DNA Marker	0.1 mL
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*IsoC = Isolateion Control; PosC= Positive Control
[a] The isolation control is a cloned PCR product
[b] The positive control is a fragment of *Parvovirus B19* cloned in a plasmid

Customer-Supplied Reagents and Equipment

- Disposable powder-free gloves
- Benchtop microcentrifuge and 60°C incubator
- Micropipettors and Sterile pipette tips with filters
- PCR tubes
- 96 – 100% ethanol

Storage Conditions and Product Stability

All buffers should be kept tightly sealed and stored at room temperature (15-25°C) for up to 1 year without showing any reduction in performance. The Parvovirus B19 2x PCR Master Mix, Control 2X PCR Master Mix, the *Isolation Control (IsoC)* and the Parvovirus B19 *Positive Control (PosC)* should be kept tightly sealed and stored at -20°C for up to 1 year without showing any reduction in performance. Repeated thawing and freezing (> 2 x) should be avoided, as this may reduce the sensitivity. If the reagents are to be used only intermittently, they should be frozen in aliquots.

General Precautions

The user should exercise the following precautions while using the kit:

- Use sterile pipette tips with filters.
- Store and extract positive material (specimens, controls and amplicons) separately from all other reagents and add it to the reaction mix in a spatially separated facility.
- Thaw all components thoroughly at room temperature before starting an assay.
- When thawed, mix the components and centrifuge briefly.
- Work quickly on ice.

Quality Control

In accordance with Norgen's ISO 9001 and ISO 13485-certified Quality Management System, each lot of Norgen's Parvovirus B19 2x PCR Master Mix, Control 2X PCR Master Mix, the *Isolation Control (IsoC)* and the Parvovirus B19 *Positive Control (PosC)* are tested against predetermined specifications to ensure consistent product quality.

Product Use Limitations

Norgen's Plasma-Serum Parvovirus B19 PCR Detection Kit is designed for research purposes only. It is not intended for human or diagnostic use.

Product Warranty and Satisfaction Guarantee

NORGEN BIOTEK CORPORATION guarantees the performance of all products in the manner described in our product manual. The customer must determine the suitability of the product for its particular use.

Safety Information

Ensure that a suitable lab coat, disposable gloves and protective goggles are worn when working with chemicals. For more information, please consult the appropriate Material Safety Data Sheets (MSDSs). These are available as convenient PDF files online at www.norgenbiotek.com.

CAUTION: DO NOT add bleach or acidic solutions directly to the sample-preparation waste.

The **Lysis Solution, Binding Solution 1** and **Binding Solution 2** contain guanidine hydrochloride, and should be handled with care. Guanidine hydrochloride forms highly reactive compounds when combined with bleach, thus care must be taken to properly dispose of any of these solutions.

If liquid containing these buffers is spilt, clean with suitable laboratory detergent and water. If the spilt liquid contains potentially infectious agents, clean the affected area first with laboratory detergent and water, and then with 1% (v/v) sodium hypochlorite.

Plasma or Serum of all human and animal subjects is considered potentially infectious. All necessary precautions recommended by the appropriate authorities in the country of use should be taken when working with plasma or serum.

1. Protocol

A. Specimen Collection, Storage and Transport

Precaution: All samples have to be treated as potentially infectious material.

1. Specimen Collection and Sample Storage

- Blood withdrawal causes injury of blood vessels (arteries, veins and capillaries).
- Only safe and sterile material should be used.
- For blood withdrawal appropriate disposables are available. For the vein puncture, too fine capillary needles should not be employed.
- Venous blood withdrawal should be carried out on the appropriate parts of the elbow bend, the forearm or the back of the hand.
- Blood has to be withdrawn with standard specimen collection tubes (red cap, Sarstedt or equivalent tube of another manufacturer). 5 - 10 ml EDTA blood should be withdrawn.

Precaution: Samples of heparinised humans must not be used

2. Sample Storage

- Whole blood should be separated into plasma and cellular components by centrifugation for 20 minutes at 800 - 1,600 x g within six hours. The isolated plasma has to be transferred into sterile polypropylene tubes.
- The sensitivity of the assay can be reduced if you freeze the samples as a matter of routine or store them for a longer period of time.
- Virus encapsulated DNA is stable for days if stored at +4°C, for weeks if stored at -20°C and even for months and years when stored at -70°C.

3. Sample Transport

- Sample material should be transported in a shatterproof, leak-proof transport container as a matter of principle. Thus, a potential danger of infection due to a leakage of sample can be avoided.
- The samples should be transported following the local and national instructions for the transport of pathogen material.
- We recommend sample transport with a courier. The blood samples should be shipped cooled (+2°C to +8°C) and the separated plasma deep frozen (-20°C).

4. Interfering substances

- Elevated levels of bilirubin (15 mg/dl) and lipids (800 mg/dl) and haemolytic samples do not influence the system.
- Heparin (10 IU/ml) affects the PCR. Samples which have been collected in tubes containing heparin as an anticoagulant should not to be used. Also, samples of heparinised patients must not be used.

B. Isolation of DNA from Plasma-Serum

Notes:

- Ensure that all solutions are at room temperature prior to use, and that no precipitates have formed. If necessary, warm the solutions and mix well until the solutions become clear again.
- Clean, disposable gloves should be worn at all times when handling reagents, samples, pipettes, disposable tubes, etc. It is recommended that gloves are changed frequently to avoid contamination
- All centrifugation steps are performed at room temperature.
- A variable speed centrifuge should be used for maximum kit performance. If a variable speed centrifuge is not available a fixed speed centrifuge can be used, however reduced yields may be observed.
- Preheat an incubator or heating block to 60°C.
- Prepare a working concentration of the **Wash Solution** by adding 50 mL of 95% ethanol (provided by the user) to the supplied bottles containing the concentrated Wash Solution. This will

give a final volume of 72 mL. The labels on the bottles have a box that may be checked to indicate that the ethanol has been added.

- Ensure that samples have not undergone more than one freeze-thaw cycle, as this may lead to DNA degradation.
- **Binding Solution 1 contains resin and must be mixed well before every pipetting**.
- **This kit is suitable for the isolation of DNA from fresh or frozen serum or plasma prepared from blood collected on either Heparin, EDTA or citrate.**
- **Isolation Control (IsoC)**
  - An Isolation Control (IsoC) is supplied. This allows the user to control the DNA isolation procedure. For this assay, add the Isolation Control (IsoC) as indicated during the isolation procedure
  - The Isolation Control (IsoC) must not be added to the sample material directly.
  - Do not freeze and thaw the Isolation Control (IsoC) more than 2 times.
  - The Isolation Control (IsoC) must be kept on ice at all times during the isolation procedure.
  - The PCR components of the Plasma-Serum Parvovirus B19 PCR Detection Kit should remain at -20°C until DNA is extracted and ready for PCR amplification.
  - It is important to work quickly during this procedure.

1. In a 2mL tube add 400 µL Plasma/Serum sample.
2. To each 400 µL Plasma/Serum sample add 1.2 mL of **Lysis Solution**. Mix well by vortexing for 15 seconds.
3. Incubate the mixture from **Step 2** for 10 minutes at 60°C.
4. After incubation add 200 µL of **Binding Solution 1** and mix well by vortexing for 15 seconds. ***(Note*: *Binding Solution 1 contains resin and must be mixed well before every pipeting*)**
5. Centrifuge the mixture from **Step 4** for **1 minute at 2,000 RPM**, then carefully decant the supernatant in order to ensure that the slurry pellet is not dislodged.
6. To the slurry pellet from **Step 5** add 180 µL from **Binding Solution 2** followed by the addition of 420 µL of **96-100% Ethanol** (provided by the user) and 10 µL **Isolation Control (IsoC)**. Mix well by vortexing for 15 seconds.
7. Centrifuge the mixture from **Step 6** for **1 minute at 2,000 RPM**, then carefully decant the supernatant in order to ensure that the slurry pellet is not dislodged.
8. To the slurry pellet from **Step 7** add 1 mL **Wash Solution**, mix well by vortexing for 15 seconds and then centrifuge for **1 minute at 2,000 RPM**. Carefully decant the supernatant in order to ensure that the slurry pellet is not dislodged.
9. Repeat **Step 8** to wash the slurry pellet for a second time.
10. To the slurry pellet from **Step 9** add 500 µL **Wash Solution,** Mix well by vortexing for 15 seconds.
11. Transfer the entire mixture from **Step 10** into a Mini Filter Spin column. Centrifuge for **1 minute at 14,000 RPM**. Discard the flowthrough and reassemble the spin column with its collection tube;
12. Spin the column, empty, for **2 minute at 14,000 RPM**. Discard the collection tube;
13. Transfer the spin column to a fresh 1.7 mL Elution tube. Apply 100 µL of **Elution Buffer** to the column and centrifuge for **2 minutes at 200 x g (~2,000 RPM),** followed by **2 minute at 10,000 x g (~14,000 RPM)**.

C. Parvovirus B19 PCR Assay Preparation

Notes:

- Before use, suitable amounts of all PCR components should be completely thawed at room temperature, vortexed and centrifuged briefly.
- The amount of PARVOVIRUS B19 2X Detection PCR Master Mix and Control 2X PCR Master Mix provided is enough for up to 32 PCR reactions (24 sample PCR, 4 positive control PCR and 4 no template control PCR).
- For each sample, one PCR reaction using the PARVOVIRUS B19 2X Detection PCR Master Mix and one PCR reaction using Control 2X PCR Master Mix should be set up in order to have a proper interpretation of the results.

- For every PCR run, one reaction containing PARVOVIRUS B19 Positive Control and one reaction as no template control must be included for proper interpretation of results.
- The recommended minimum number of DNA samples tested per PCR run is 6.
- Using a lower volume from the sample than recommended may affect the sensitivity of the PARVOVIRUS B19 Limit of Detection.

1. Prepare the PCR reaction for sample detection (Set #1, using **PARVOVIRUS B19 2X Detection PCR Mastermix**) and the PCR reaction for control detection (Set #2, using **Control 2X PCR Mastermix**) as shown in Table 1 below. The recommended amount of sample DNA to be used is 2.5 µL. However, a volume between 1 and 5 µL of sample DNA may be used as template. Ensure that one PARVOVIRUS B19 detection reaction and one control reaction is prepared for each DNA sample. Adjust the final volume of the PCR reaction to 20 µL using the Nuclease-Free Water provided.

Table 1. PCR Assay Preparation

PCR Components	Volume Per PCR Reaction
PARVOVIRUS B19 2X PCR Master Mix OR Control 2X PCR Master Mix	10 µL
Sample DNA	2.5 µL
Nuclease-Free Water	7.5 µL
Total Volume	20 µL

2. For each PCR set, prepare **one** positive control PCR as shown in Table 2 below:

Table 2. PCR Positive Control Preparation

PCR Components	Volume Per PCR Reaction
PARVOVIRUS B19 2X PCR Master Mix OR Control 2X PCR Master Mix	10 µL
PARVOVIRUS B19 Positive Control (PosC)	10 µL
Total Volume	20 µL

3. For each PCR set, prepare **one** no template control PCR as shown in Table 3 below:

Table 3. PCR Negative Control Preparation

PCR Components	Volume Per PCR Reaction
PARVOVIRUS B19 2X PCR Master Mix OR Control 2X PCR Master Mix	10 µL
Nuclease-Free Water	10 µL
Total Volume	20 µL

D. PARVOVIRUS B19 PCR Assay Programming

1. Program the thermocylcer according to the program shown in Table 4 below.
2. Run one step PCR.

Table 4: PARVOVIRUS B19 PCR Assay Program

PCR Cycle	Step	Temperature	Duration
Cycle 1	**Step 1**	**95°C**	**3 min**
Cycle 2 (40x)	**Step 1**	**94°C**	**15 sec**
	Step 2	**60°C**	**30 sec**
	Step 3	**72°C**	**45 sec**
Cycle 3	**Step 1**	**72°C**	**5 min**
Cycle 4	**Step 1**	**4°C**	**∞**

E. Parvovirus B19 PCR Assay Interpretation

- For the analysis of the PCR data, the entire 20 µL PCR reaction should be loaded on a 1X TAE 2% Agarose DNA gel along with 10 µL of Norgen's DNA Marker (provided).
- The PCR products should be resolved on the 1X TAE, 2% Agarose gel at 150V for 30 minutes (Gel running time will vary depending on an electrophoresis apparatus).

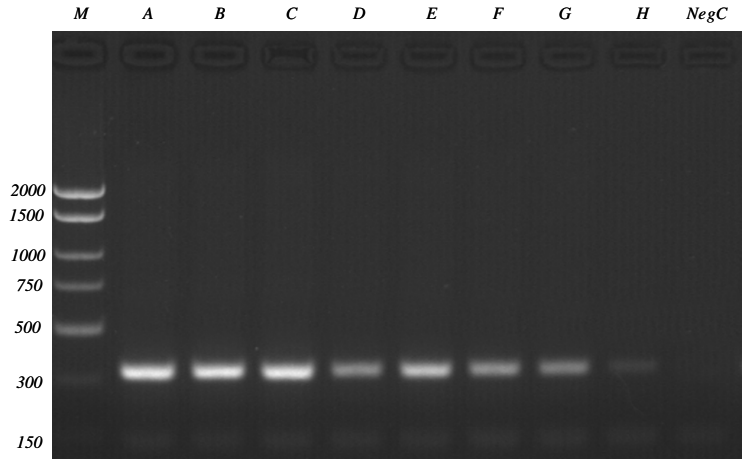

Figure 1: A representative 1X TAE, 1.7% agarose gel showing the amplification of Parvovirus B19 at different concentrations (*Target*). The size of the Parvovirus B19 target amplicon corresponds to the 322bp band represented by the provided DNA Marker (M). Lanes A-H represents samples spiked with different *Parvovirus B19*. Neg corresponds the negative control (Nuclease-Free Water).

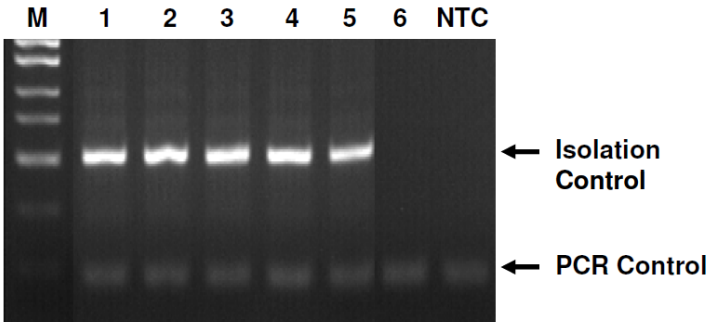

Figure 2: A representative 1X TAE 1.7% agarose gel showing the amplification of Isolation Control and PCR Control under different conditions using the Control 2X PCR Master Mix. The size of the Isolation Control amplicon and PCR Control amplicon correspond to 499 bp and 150 bp, respectively, as represented by the provided DNA Marker (M). Lanes 1 to 5 showed detection of both Isolation Control and PCR Control, suggesting that the DNA isolation as well as the PCR reaction was successful. Lane 6 showed only the detection of PCR Control suggesting that while the PCR was successful, the isolation failed to recover even the spiked-in Isolation control. NTC=Negative Control.

Table 5. Interpretation of PCR Assay Results

Input Type	Target Reaction	Control Reaction		Interpretation
	PARVOVIRUS B19 Target Band (322 bp)	IsoC Band (499bp)	PCRC Band (150 bp)	
Positive Control	X	X	X	Valid
Negative Control			X	Valid
Sample	X	X	X	Positive
Sample		X	X	Negative
Sample		X		Negative
Sample			X	Re-Test
Sample				Re-Test
Sample	X	X		Positive
Sample	X		X	Positive
Sample	X			Re-Test

** For results obtained that are not covered in Table 5 above, please refer to the Troubleshooting Section.

** Ignore any bands that appear between the Isolation Control band and the PCR Control band

F. Specificity

- The specificity of Norgen's Plasma-Serum Parvovirus B19 PCR Detection Kit is first and foremost ensured by the selection of the Parvovirus B19-specific primers, as well as the selection of stringent reaction conditions. The primers were checked for possible homologies in GenBank published sequences by sequence comparison analyses.

G. Linear Range

- The linear range (analytical measurement) of Norgen's Plasma-Serum Parvovirus B19 PCR Detection Kit was determined by analyzing a dilution series of Parvovirus B19 quantitative standard ranging from 8.46×10^{9} VP/µl to 1×10^{-1} IU/µl.
- Each dilution has been tested in replicates (n = 4) using Norgen's Plasma-Serum Parvovirus B19 PCR Detection Kit on 1X TAE, 1.7% Agarose gels.
- The linear range of Norgen's Plasma-Serum Parvovirus B19 PCR Detection Kit has been determined to cover concentrations from 10 VP/µl to at least 8×10^{6} VP/µl
- Under the conditions of Norgen's Plasma-Serum DNA Isolation procedure, Norgen's Plasma-Serum Parvovirus B19 PCR Detection Kit covers a linear range from 1000VP/mL Plasma-Serum to at least 8×10^{9} VP/mL Plasma-Serum.

H. Frequently Asked Questions

1. How many samples should be included per PCR run?

- Norgen's Plasma-Serum PARVOVIRUS B19 PCR Detection Kit is designed to test 24 samples. For every 6 samples, a Negative Control and a Positive Control must be included. It is preferable to pool and test 6 samples at a time. If not, the provided Negative Control and Positive Control are enough to run 3 samples at a time.

2. How can I interpret my results for a sample if neither the PARVOVIRUS B19 PCR Control nor the PARVOVIRUS B19 Isolation Control (IsoC) amplifies?

- If neither the PARVOVIRUS B19 PCR control nor the PARVOVIRUS B19 Isolation Control (IsoC) amplifies, the sample must be re-tested. If the positive control showed amplification, then the problem occurred during the isolation, where as if the Positive control did not amplify the problem has occurred during the setup of the PCR assay reaction.

3. How should it be interpreted if only the PARVOVIRUS B19 PCR control showed amplification but neither the PARVOVIRUS B19 target nor the PARVOVIRUS B19 Isolation Control (IsoC) amplified for a sample?

- This indicates a poor isolation. The isolation procedure must be repeated.

4. How should it be interpreted if only the PARVOVIRUS B19 Isolation Control (IsoC) was amplified in a sample?

- The sample tested can be considered as PARVOVIRUS B19 negative.

5. How should it be interpreted if only the PARVOVIRUS B19 target and the PARVOVIRUS B19 PCR control were amplified in a sample?

- The sample tested can be considered as PARVOVIRUS B19 positive.

6. How should it be interpreted if only the PARVOVIRUS B19 target was amplified in a sample?

- The sample tested can be considered positive. At PARVOVIRUS B19 viral load, the PARVOVIRUS B19 amplicon will be predominant and the PARVOVIRUS B19 PCR control as well as the PARVOVIRUS B19 Isolation control may not amplify.

7. How should it be interpreted if only the PARVOVIRUS B19 PCR control and the PARVOVIRUS B19 Isolation Control (IsoC) showed amplification?

- The sample tested can be considered negative

8. Can I process a different Plasma-Serum volume?

- The reagents provided with the isolation kit are only sufficient to process 24 Plasma-serum samples of 0.5mL each.

9. What If I added more or less of the specified reagents' volume?

- Adding less volume may reduce your DNA yields. Adding more may not affect the DNA yields EXCEPT if more Elution Buffer was added. Eluting DNA in higher volumes of Elution Buffer will result in diluting your DNA.

10. What If my incubation varied from the 10 minutes specified in the product manual?

- Less than 10 minutes will result in lower DNA yields. More than 10 minutes may not affect your DNA yields.

11. What If I forgot to do a dry spin after my second wash?

- Your first DNA elution will be contaminated with the Wash Solution. This may dilute the DNA yield in your first elution and it may interfere with your down stream applications.

12. What If I forgot to add the PARVOVIRUS B19 Isolation Control (IsoC) during the isolation?

The isolation must be repeated.

Technical Assistance

NORGEN's Technical Service Department is staffed by experienced scientists with extensive practical and theoretical expertise in sample and assay technologies and the use of NORGEN products. If you have any questions or experience any difficulties regarding Norgen's Plasma-Serum Parvovirus B19 PCR Detection Kit or NORGEN products in general, please do not hesitate to contact us.

NORGEN customers are a valuable source of information regarding advanced or specialized uses of our products. This information is helpful to other scientists as well as to the researchers at NORGEN. We therefore encourage you to contact us if you have any suggestions about product performance or new applications and techniques.

For technical assistance and more information, please contact our Technical Support Team between the hours of 8:30 and 5:30 (Eastern Standard Time) at (905) 227-8848 or Toll Free at 1-866-667-4362 or call one of the NORGEN local distributors (www.norgenbiotek.com) or through email at techsupport@norgenbiotek.com.

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